REMARKS

Rejection of Claims and Traversal Thereof

In the August 26, 2009 Office Action:

1. Claims 1, 4-10, 12-16 and 18-22 and 24-27 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lockhart et al., (WO 97/27317), in view of Lakowicz et al., (Photon Spectra (October 2001) 35(10): 96-104 (The Office needs to correctly identify this reference, wherein the lead author is Lakowicz and not Gryczynski,et al.); and in further view of Cao, et al., (Journal of the Amer. Chem. Soc. (July 2001) 123: 7961-7962 and in further view of Qi et al., (Applied and Environmental Microbiology (2001) 67(8): 3720-3727; and

2. Claims 1, 4-10, 12-16 and 18-22 and 24-27 were rejected under 35 U.S.C. 103(a) as being unpatentable over Cao, et al., (Nanoparticles within Raman Spectroscopic Fingerprints for DNA and RNA Detection, Science, Aug 2002, Vol. 297, pp 1536-1540, hereinafter Cao); as evidenced by Malicka,et al., (Biopolymers (2003) 72(2) 96-104, hereinafter Malicka) and Lukomska et al., (Biopolymers and Biophysical Research Communication (2005) 328: 78-84) in view of Lakowicz 1 (US Patent Application No. 2002/0160400), and in further view of Lakowicz 2, (Radiative Decay Engineering: Biophysical and Biomedical Applications," Analytical Biochemistry, 2001, Vol. 298, pp 1-24, hereinafter Lakowicz 2).

These rejections are hereby traversed and reconsideration of patentability of the pending claims is therefore requested in light of the following remarks.

Rejections under 35 U.S.C. 103 (a)

Claims 1, 4-10, 12-16 and 18-22 and 24-27 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lockhart et al., in view of Lakowicz et al., Cao, et al., and Qi et al. Applicants submit that the proposed combination does not in any way, disclose, teach or suggest the presently claimed invention.

The Office mistakenly believes that Lockhart describes all the limitations of claim 1 excepting the positioning of the probes to on the metal particles, positioning of the fluorophore near the metallic

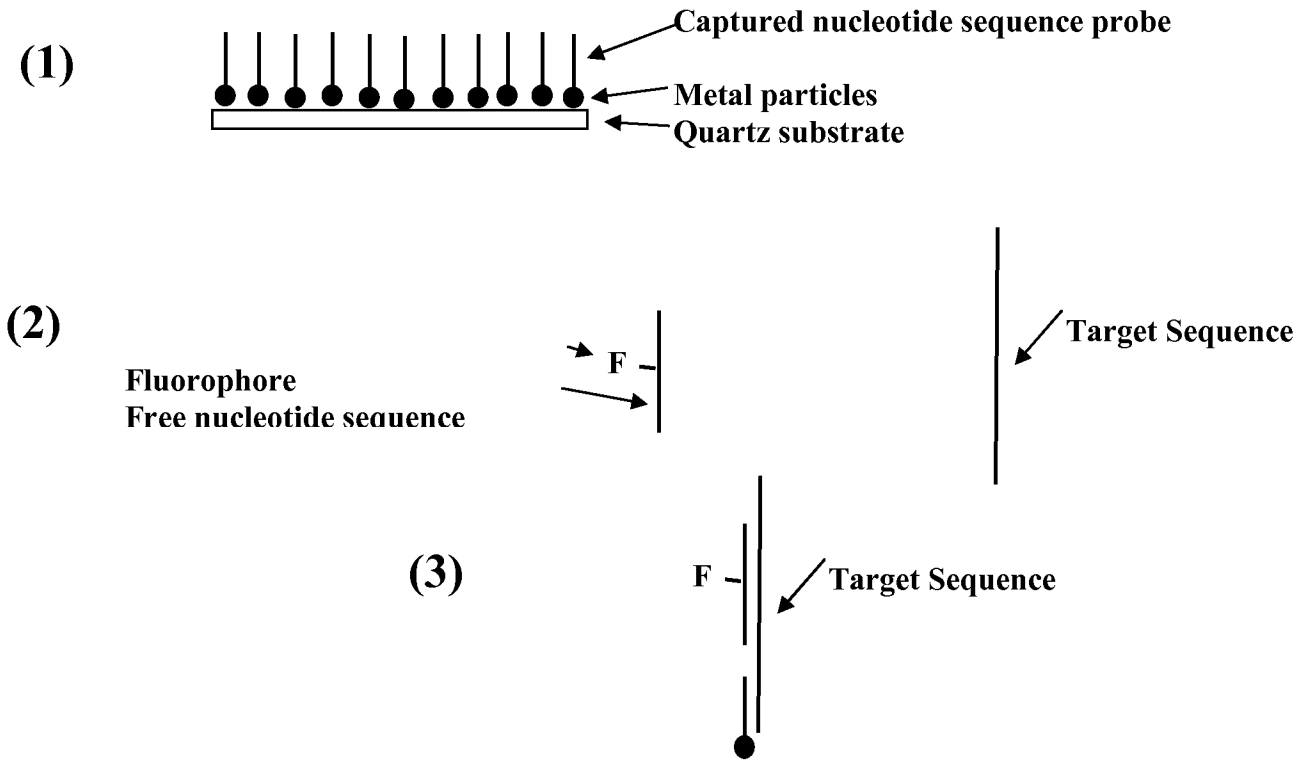
surfaces and the detection of Baccillus anthracis. Applicants vigorously disagree because Lockhart alone or in combination with the other references does not teach the components of the presently claimed invention.

Applicants' claimed invention as recited in claim 1 include:

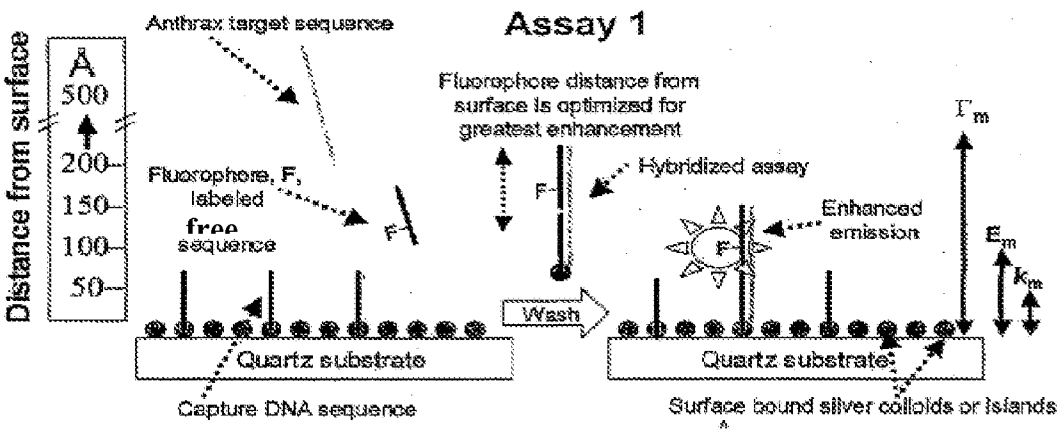
(1) a substrate with immobilized metal particles, wherein the metal particles include a covalently bonded captured nucleotide sequence probe complementary to a first known sequence of a nucleotide sequence of the *B. anthracis*, and

(2) a free nucleotide sequence probe, wherein the free nucleotide sequence probe has been fabricated to a second known sequence of the *B. anthracis* and having an affinity for said nucleotide sequence of *B. anthracis*, wherein a fluorophore is attached to the free nucleotide sequence and when the free nucleotide sequence hybridizes with the known sequence of the *B. anthracis* the fluorophore is positioned from about 50 to 500Å from the metallic surface,

(3) both of these two probes are necessary to determine if a test sample includes the nucleotide sequence of *B. anthracis.*



It should be very clear that the nucleotide sequence of *B. anthracis* is known so the two probes include sequences that are complementary to two different regions of the **known anthrax sequence** and are **prepared to attach to different regions of the *B. anthracis* sequence**. Clearly if there is no *B. anthracis* sequence in the sample then the captured nucleotide sequence probe will remain unbound, and thus, the free probe will not bind to anything and in turn–no signal. In the alternative, if there is *B. anthracis* sequence in the sample then it will attach to the captured nucleotide sequence probe and then the free nucleotide sequence probe will bind to the second site that is complementary to the free nucleotide sequence probe and a signal produced. Thus applicants' system includes the use of two separate and distinct probe sequences (a captured and free nucleotide sequence) that are complementary to known different sections of the target nucleotide sequence. The free nucleotide probe sequence includes a fluorophore positioned a distance from the metal surface. This is shown below by applicant's Figure 1.



Importantly, the use of the two probes that have affinity for different nucleotide sequences in the target sequence, under highly stringency conditions, is advantageous because it allows for increased sensitivity. Thus, when both probes are bound to the target sequence there is very little doubt regarding the identity of the bound sequence. In fact using the two probes provides for additional verification that the target sequence is indeed anthrax.

Originally the Office made reference to several sections of the Lockhart reference and specifically page 71 and Figures 12 and 13. In this section relating to Figures 12 and 13, the reference describes an array containing a large number (greater than a 1000) of arbitrarily selected different oligonucleotide probes where the sequence of the probe is known and the exact location in the assay is known. Thus, there is a multiplicity of different capture probes. This large number of probes is essential so that differences in the hybridization patterns can be used to determine the differences in the expression of various genes.

In the current Office Action, the Office has changed its view because applicants pointed out that the capture probe sequences are a single nucleotide sequence that is complementary to the known target sequence. In light of the lesson by applicants explaining the present invention to the Office, the Office went back to the Lockhart reference searching for similar terminology. Yes, the Office found a few sections in the 200 page reference relating to known target sequences but there were also numerous different types of probes that were used to hybridize to the numerous target sequences.

This reference provides a multiplicity of different options and "homing-in" on certain sections of this reference could only be accomplished with the use of applicants' specification as a road map during the hunting expedition. The reference provides numerous different types of attached probes to a surface including probes that can include a constant region and a variable region, probes that are complementary to the nucleic acid, probes that are not complementary to the nucleic acid in sample, probes that are random, haphazard, and all possible oligonucleotides of a preselected length, pair of probes wherein each pair differ from each other in preselected nucleotides, etc. It is very clear that Lockhart teaches the use of an array of different probes and states on page 51 that the probes can be random, arbitrary haphazard, composition biased or include all possible oligonucleotides of a particular length. Further on page 53, the reference states that the invention can include 1,000,000 different probes, to provide every probe of a characteristic length that binds to a particular nucleic acid sequence. On page 71, there is a discussion that the probes can include a constant region but if they do they MUST also include a variable region which again provides for the required randomness.

Further, regarding labeling of the target nucleic acid in Lockhart, the labeled probes must include a ligatable oligonucleotide and ligase. For determination of the labeled sequence, there must be a ligase involved so that the probe and labeled nucleotide sequence can be ligated together as shown in Figure 12, and recreated below.

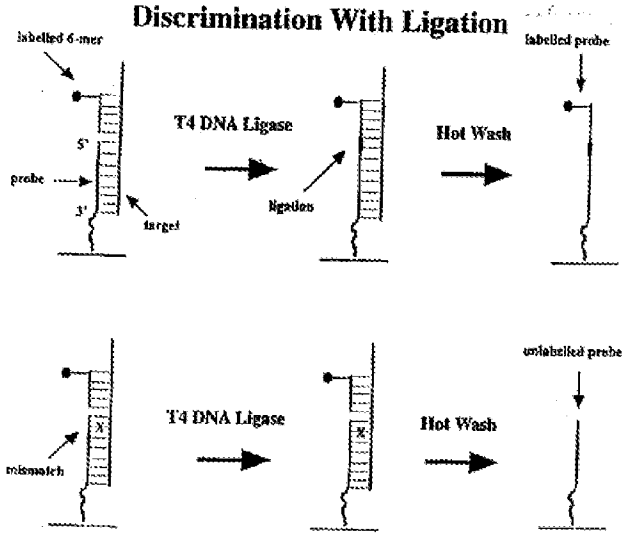


Fig. 12

Thus, the two sequences have to be sufficiently close to allow such ligation and it is very apparent the label must be at the end of 5' end of the ligatable oligonucleotide. Clearly, there is nothing in this reference that discloses or even recognizes the importance of placement of the label for interaction with metallic particles on the substrate.

Still further, Lockhart provides for labels attached to the 5' terminus end of a nucleotide sequence and thus with all the possible lengths of the probes, there is no chance of continuity or the possible placement of a fluorophore at a specific distance from a metallic particle. The present invention demands continuity, that being, a single probe that has affinity for a single sequence area on the target pathogen which will allow the second free probe to attach at the optimal position.

The Office realizes that Lockhart does not provide for any teaching relating to the use of metal particles or metallic surface and does not teach sandwiching a fluorophore between a metal colloid and a metal surface. What the Office has completely overlooked is that the Lockhart also does not teach the placement of a fluorophore at a specific placement on a free nucleotide sequence so that it is positioned at an optimal length from the metallized surface.

According to the Office, Lakowicz (wrongly recited as Gryczynski in numerous office actions) teaches a method for increasing the fluorescence of a fluorophore by using metal particles. However, the Lakowicz reference does not provide any indication of placement of a fluorophore on a nucleotide sequence that provides any guidance to go in the direction of applicants' claimed invention. Instead, there is a discussion of intrinsic DNA fluorescence molecules that do not include an external fluorophore. The only discussion relating to the addition of an extrinsic fluorophore to the DNA relates to the negative aspects of such extrinsic fluorophores as discussed on page 101 at the bottom of column 1 of the Lakowicz reference, wherein the text expressly states that using extrinsic fluorophores introduces complications including the limiting factor of having to label the DNA.

Instead the reference uses the intrinsic method and as discussed in Figure 6, recreated above, only **unlabeled nucleotides are used**. Thus, it is evident that the Lakowicz reference does not provide any information regarding labeling a DNA nucleotide probe with a fluorophore that is positioned a specific distance from metallic particles.

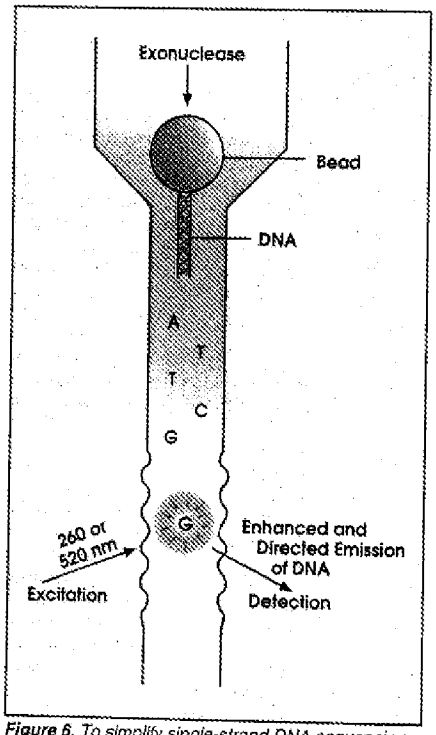


Figure 6. To simplify single-strand DNA sequencing, metal particles could enhance the intensity and provide directionality for emissions of unlabeled nucleotides.

Increased intrinsic emission from DNA may provide new approaches to DNA analysis.

One possibility is single-strand DNA sequencing.[8] The goal is to use exonuclease cleavage of terminal DNA bases that are identified after their sequential release.

A limiting factor in this approach will be the reactive yield of labeling each nucleotide with an extrinsic fluorophore after its release.

Instead, an appropriately designed flow chamber employing metallic particles could enhance the base emission by a combination of the lightning-rod effect, increased radiative rate, decreased lifetime and increased photostability, all contributing to more photons per released base (Figure 6).

As stated above, Lockhart provides for labels attached to the 5' terminus end of a nucleotide sequence but Lakowicz teaches that additional extrinsic fluorophores are a problem and instead uses DNA as an intrinsic fluorophore. There is **nothing** in Lockhart that that teaches the use of **intrinsic fluorophore, that being just the DNA having intrinsic fluorescence.** One skilled in the art would never consider using the teaching of Lakowicz in combination with the Lockhart system because it is very evident that the Lockhart system would no longer operate as intended or it could change the mode of operations. Clearly, the label of Lockhart is essential to determine if the ligase provided the necessary ligation and if the label is not there, then the expected hybridization did not occur.

The MPEP § 2143.01 V – VI states that:

> "If proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to

> make the proposed modification. … [and] If the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims prima facie obvious."

Further, according to the court in *In re Gordon,* 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984), if proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification and the Office has not established a *prima facie* case of obviousness.

Thus, reduced to the basics, Lockhart teaches a multiplicity of different probes attached to a glass substrate but never mentions the use of metal particle on a surface and never considered placing a fluorophore on a free nucleotide sequence at an optimal distance from the metallic particles. The only label used by Lockhart includes placement at the end of the 5'end terminus of a sequence. The Lakowicz reference does not even use a fluorophore and in fact discourages the use of same. Further, there are no probe sequences attached to any metal surfaces. Instead DNA is passed through a tube that has an inner surface with metal particles wherein the DNA with some intrinsic fluorescence exhibited intensified fluorescence. In conclusion the combination of Lockhart and Lakowicz does not teach or suggest a detection system comprising a surface substrate with metallic particles and a capture probe sequence attached to the metallic particles, wherein the capture probe can be hybridized to a target sequence. Further this combination does not teach the use of a free sequence probe that once attached to the target sequence positions a fluorophore the optimal distance from the metallic particles to enhance the fluorescence of the fluorophore in a detection method.

The Office has cited several other references in an attempt to establish a *prima facie* case of obviousness, however the addition of Cao or Qi does not rectify the shortcomings of the Lockhart and Lakowicz combination regarding claim 15.

Cao teaches the use of silver nanoballs that are coated with gold to provide the gold plasmonic signals in the use of raman spectroscopy but avoids the downside of silver particles that tend to degrade in DNA hybridization environments. Notably the silver/gold coated nanoballs are free in solution and can be used as a label technique. However, even with this additional piece there is no guidance in any of the references for a detection system comprising a surface substrate with

metallic particles and a capture probe sequence attached to the metallic particles, wherein the capture probe can be hybridized to a target sequence. Further this combination does not teach the use of a free sequence probe that once attached to the target sequence (and attached to the capture probe) positions a fluorophore the optimal distance from the metallic particles to enhance the fluorescence of the fluorophore in a detection method.

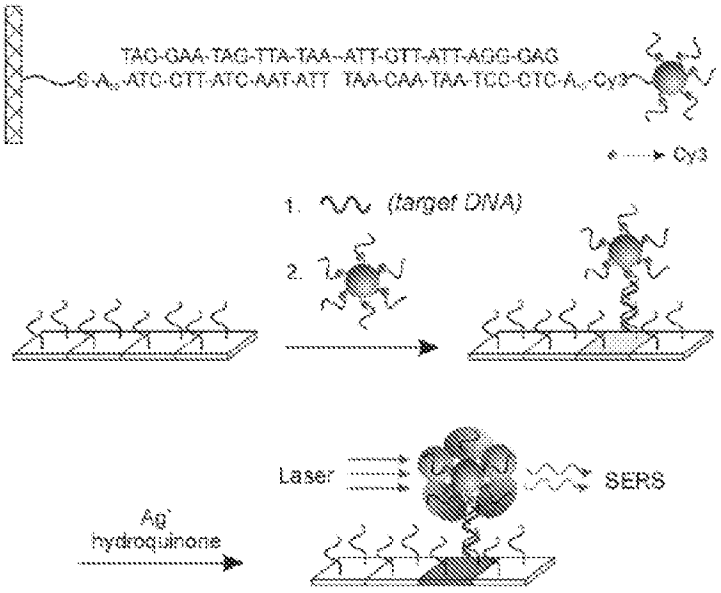
Qi is cited because it is the final piece of the puzzle, that being, the mere mention of Bacillus anthracis. However, if none of the prior art teaches or suggests all the claimed components then the prior art does not defeat the patentability of the pending claims. Applicants request the withdrawal of this rejection under section 103.

2. Claims 1, 4-10, 12-16 and 18-22 and 24-27 were rejected under 35 U.S.C. 103(a) as being unpatentable over Cao as evidenced by Malicka and Lukomska in view of Lakowicz 1 and 2. Once again applicants insist that the proposed combination does not defeat the patentability of the presently claimed invention.

Cao describes the use of gold nanoparticles combined with surface-enhanced Raman scattering spectroscopy for detection and identification of single dye molecules. Cao expressly states that they use this method to overcome the problems related to using molecular fluorophores because of overlapping spectral features and non uniform fluorophore photobleaching rates which could lead to potential complications. Cao designed a probe that is built around a 13 nm gold nanoparticle that is functionalized with Raman dye-labeled oligonucleotides. The nanoparticles are coated with hydrophilic oligonucleotides containing a **Raman dye at one end** and terminally capped with a small molecule recognition element (e.g. biotin). After the probe is attached to a small molecule or an antigen it is designed to detect, the substrate is exposed to silver and hydroquinone solution. The silver-plating grows around the Cy3-labeled probes leading to raman scattering occurring in close proximity to the Raman dye, which allows for dye signature detection with a standard Raman microscope.

Notably Cao uses a surface substrate (**such as glass with no metal coating**) that is coated with nucleotide sequences. This surface substrate with the nucleotides attached thereto is kept in **a humidity chamber** at room temperature and then is contacted with oligonucleotide functionalized nanoparticles that include the following (1) Au nanoparticles, (2) a DNA sequence

complimentary to the surface attached nucleotide sequences and also has attached a Cys3 label. Ag containing solution is then added to the assay to increase the size of the metal presence of the Au metal colloid as shown below from Cao:



Notably, this additional of silver ions is attached to the Au particle to grow around the Cy3-labeled Au probe to increase it size to provide for raman scattering. Clearly, the increase in size of the Au particle is of great concern because in fact if the gold particle is too large it has the ability to quench any signal from a fluorophore, so clearly there is no incentive to view the teaching of Cao as credible guidance to go in the direction of applicants' claimed invention

Just by reviewing the figure above from Cao it is very evident that the raman dye is always placed adjacent to the gold nanoparticle. Clearly this reference is not concerned about a specific enhancement positioning of a fluorophore relative to a substrate surface comprising immobilized metallic nanoparticles.

According to the Office, Cao does not teach that immobilized capture probes are immobilized to metal particles on a substrate and that Lakowicz teaches a method for increasing fluorescence intensity of a fluorophore using metal particles. Applicants insist that Lakowicz 1 does not provide any guidance for applicants' invention and certainly does not rectify the shortcomings of Cao.

Relating to the use of DNA or RNA, the Lakowicz 1 reference is limited to the use of two (2) nucleotides sequences. One that is attached to a metalized substrate that includes a fluorescence label and then another complementary sequence, as discussed in column 2, page 7, paragraphs [0088] to [0090]. Thus, the probe with fluorescence tag is immobilized on the metallized surface substrate and when another nucleotide sequence is attached to the immobilized probe, the reference states that the fluorescence is enhanced. In another embodiment, the immobilized probe has a donor molecule and a complementary sequence has an acceptor molecule, thus when the hybridization occurs there is a transfer of energy between the donor and acceptor molecule. Importantly, this reference never discusses the use of a sandwich assay system in combination with nucleotide sequences wherein a captured nucleotide sequence probe is attached to a metallized surface substrate and has the ability to capture a target sequence with a known nucleotide sequence. Then a free nucleotide sequence probe can bind to the known sequence, if it has been attached, and the free nucleotide sequence comprises a fluorescence tag that is positioned at the correct placement on the free nucleotide so that when irradiated, the emissions are enhanced because of the correct proximity to the metallized substrate.

Importantly, these two references, that being Cao and Lakowicz 1, teach entirely different methods and concepts and clearly there is no suggestion to modify either reference. The important aspects of Cao include the gold nanoballs attached to the Cys dye and positioned at the opposite end of the substrate. Then the entire setup is bathed in a silver solution to provide a signal. Clearly, looking at the Figures of Cao, the bathing in the silver solution is important and there is no signal without this bathing. Notably, the Cao dye label is not bathed in silver until after hybridization with a complementary sequence but the exact opposite is true for Lakowicz wherein the label is attached to a sequence and then hybridization occurs. Further, the metallic particles are in entirely different spaces, the Lakowicz is on the surface of substrate and the Cao is in free space.

Under Graham, and as required by MPEP §§ 2111 and 2141.02, the Office must ascertain the differences between the claimed invention and the prior art, and must consider both the invention and **the prior art as a whole**. Thus, even in light of the *KSR* decision, **the Office must consider the inventions of any cited references in their respective entireties.** Certain individual features from the references may not be arbitrarily chosen (while equally arbitrarily discarding other disclosed features) to merely lump together disparate features of different references as a mosaic in

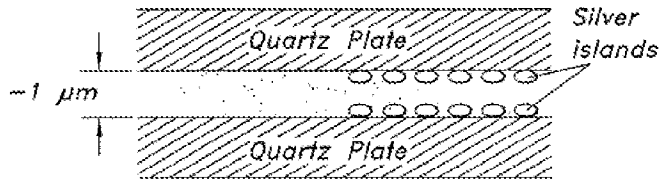
an attempt to meet the features of the rejected claims. Thus, the Office is not allowed to pick and choose just certain parts of different references and combine them, **but instead, the references in their entirety must be considered.**

Further, applicant reminds the Office that Section 2143.01 of the MPEP, as well as the ruling in *In re Ratti*, (270 F.2d 810 (CCPA 1959)) state that where a proposed modification or combination would change the **principle of operation of the prior art invention being modified**, then the teachings are not sufficient to establish a *prima facie* case of obviousness. Thus, a combination of references that fundamentally change the "basic principals" under which the prior art was designed to operate cannot support a finding of obviousness. According to the Board in *Ex Parte Vito Cellini*, (Appeal 2008-4104, BPAI 2008), "a change in the basic principles" refers to change that is fundamental in scope so as to **relates to scientific or technical principles of operation.**

Applicants insist that the suggested combination of Cao and Lakowicz will change the "basic scientific principles" of both cited references for the following reasons. Cao describes a critical step that includes the use of silver solution to add to the circumference of the gold nanoball before irradiation. However, if this silver solution is added to Lakowicz, then the silver particles will increase and the signal will likely be quenched. If the silver solution is left out of Cao it will not work and certainly will not gain anything by placing a silver coating on the substrate such as Lakowicz because the raman dye needs to be adjacent to the gold nanoball. Further, there are numerous issues relating to use of fluorophore or raman dye because Cao complains about fluorophores. Clearly there is no guidance for picking and choosing from either reference to go in the direction of applicants' claimed invention unless of course the Office is again using the applicants' specification as a blueprint for a hunting expedition, which we all know is considered to be impermissible hindsight.

Thus, the suggested combination of references would require a substantial change in the elements of the prior art as well as a change in the basic scientific principals under which the prior art was designed to operate. As such, the proposed combination does not establish a *prima facie* case of obviousness because the combination of references fundamentally change the "basic principals" under which the prior art was designed to operate.

Notably, if one combines Lakowicz 2 with Cao and Lakowicz 1, the proposed combination will suffer from the same shortcomings. Lakowicz 2 describes a system that includes two quartz plates with silver islands and DNA was placed between the two plates, as shown below:



Notably, there are no fluorophores used in this system but instead the authors are showing that DNA has the ability to provide intrinsic fluorescence that can be enhanced by metal particles. Notably, there is no discussion in this entire reference relating to optimal placement of a fluorophores or even capturing a target DNA.

Thus, even with the proposed combination there is no teaching or suggestion for all the presently claimed limitations as set forth in the present claims. In light of the foregoing discussion and the fact that all of claimed limitations are not disclose it is clear that the Office has not met its burden of establishing a *prima facie* case of obviousness.

Multiplicity of References

Applicants question the need for so many cited references in an attempt to establish a *prima facie* case of obviousness. Applicants submit that if the Office had a soundly based position on the issue of obviousness, it would not be necessary to rely on so many references. In this regard, the comments of the Board in *Ex Parte Blanc*, 13 USPQ2d 1383 (B.P.A.I. 1989) citing *Ball & Roller Bearing Co. v. F.C. Sanford Mfg. Co.*, 297 F. 163 (2d Cir. 1924) seem particularly pertinent. The Board stated that:

> "It seems necessary to apply to patent litigation from time to time the maxim that one cannot make omelettes of bad eggs--no matter how many are used. One good reference is better than 50 poor ones; and the 50 do not make the one any better."

Other Courts have agreed and have noted that the reliance on a large number of references is persuasive evidence of invention. See, e.g., *Handy v. American Flyer Mfg. Co.*, 6 USPQ 294 (S.D.N.Y. 1930) which stated that:

> "And the citation of many prior references, none showing a solution of the problem presented, is persuasive evidence of invention."

To date the Office has cited the followings references including the five set forth below and cited by the first examiner assigned to the examination of this application:

1. Vo Dinh US Patent No. 5,814,516;
2. Lakowicz, et al. (US 2002/0160400);
3. Carron et al, US Patent No 6,770,488;
4. Lakowicz, "Radiative Decay Engineering: Biophysical and Biomedical Applications." Analytical Biochemistry, 2001 V. 298, PP 1-24;
5. Cao, "Nanoparticles within Raman Spectroscopic Fingerprints for DNA and RNA Detection, Science, Aug. 2002, V. 297, pp1536-1540;

Another examiner was appointed to this application and instead of giving full faith and credit to the previous examination, the new examiner conducted a completely new search in total disregard of Section 704.01, as set forth:

704.01 Search - 700 Examination of Applications

704.01 Search

After reading the specification and claims, the examiner searches the prior art. The subject of searching is more fully treated in MPEP Chapter 900. See especially MPEP § 904 through § 904.03. The invention should be thoroughly understood before a search is undertaken. However, informal cases, or those which can only be imperfectly understood when they come up for action in their regular turn are also given a search, in order to avoid piecemeal prosecution.

PREVIOUS EXAMINER'S SEARCH

When an examiner is assigned to act on an application which has received one or more actions by some other examiner, full faith and credit should be given to the search and action of the previous examiner unless there is a clear error in the previous action or knowledge of other prior art. In general the second examiner should not take an entirely new approach to the application or attempt to reorient the point of view of the previous examiner, or make a new search in the mere hope of finding something. See MPEP § 719.05.

Clearly in light of the fact that only two of the above references has been recited by the newly appointed examiner, the present examiner did not review the guideline in the MPEP and instead conducted a further search and citing the following group of references.

6. Lockhart, WO 97/27317;

8. Lakowicz et al, Photonic Spectra 2001 V. 35 PP96-104;

9. Cao et al., Journal of American Chemical Society 2001, B.123, pp. 7961-7962;

10. Qi, et al., Applied and Environmental Microbiology 20021, V. 67, pp.3720-3727;

11. Malicka et al, (Biopolymer 2003 V. 72, pp 62-104;

12. Lukomska et al, Biochemical and Biophysical Research Comm. 2005, V. 328, pp. 78-84; and

13. Lakowicz, et al., Biochemical and Biochemical Research Communications 2001 V. 286, 875-879.

Applicants contend that the use by the Office of a multiplicity of references in an effort to show obviousness is in itself persuasive of the futility of attempting to establish a *prima facie* case of obviousness.

Fees Payable

No fee is due for entry of this response, however, if any additional fee is found due for entry of this amendment, the Commissioner is authorized to charge such fee to Deposit Account No. 13-4365 of Moore & Van Allen.

Conclusion

Applicants have satisfied the requirements for patentability. All pending claims are free of the art and fully comply with the requirements of 35 U.S.C. §112. It therefore is requested that Examiner Bertagna reconsider the patentability of the pending claims in light of the distinguishing remarks herein, and withdraw all rejections, thereby placing the application in condition for allowance. If any issues remain outstanding incident to the allowance of the application, Examiner Bertagna is requested to contact the undersigned attorney at (919) 286-8089.

Respectfully submitted,

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